99. (New) The mammal of claim 88, wherein said protein or peptide is selected from the group consisting of prothrombin, Factor VII, Factor IX, Protein C, Protein S, Factor V, Factor VIII, α1-anti-trypsin, antithrombin III, fibrinogen, albumin, an immunoglobulin, a hormone, a growth factor, erythropoietin, a bone morphogenetic protein and an ion channel protein.

D1 CONT.

100. (New) The mammal of claim 88, wherein said transgenic animal is a pig, sheep, goat, cow, rodent, rabbit or horse.

REMARKS

Upon entry of this amendment, claims 75-100 are pending. Claims 5-7, 11-13, 15, 45-47, 51-65 and 67-74 have been canceled without prejudice or disclaimer and new claims 75 – 100 have been added. Applicants reserve the right to file any canceled subject matter or claims in one or more continuing applications. The new set of claims is supported by the specification on page 30, line 17 to page 31, line 3 and throughout the specification. Particularly claims 78-83 and 91-96 are supported on page 42. The Examiner states that the claims 5, 15, 52, 53, 62, 63, 68, 70, 72 and 74 appear to be free of the prior art . The new set of claims is intended to more clearly define the claimed invention and is not intended to limit the scope of the invention.

1. Claim Objections

The Examiner objects to claims 45 and 56 as containing the term "sequencesoperably." New claims 75 and 88 that correspond to previous claims 45 and 56 have been amended to correct this typographical error.

2. Rejections under 35 U.S.C. 112, first paragraph

Claims 70 and 74

Claims 70 and 74 are rejected under 35 U.S.C. 112, first paragraph, as allegedly not being described in the specification. Applicants respectfully disagree with the Examiner's position that the phrase "3' urinary tract-specific" sequences is not supported in the specification. Applicants refer the Examiner to pages 42, lines 12-14 where it is recited that the 3' regulatory sequences useful in the present invention result in the expression of

the DNA sequence in the urinary tract cells of the animal. Applicants have amended the claims to utilize this language. It is requested that this rejection be withdrawn.

Claims 5-7, 11-13, 15, 45-47, 51-57, 61-65 and 67-74

The Examiner rejected all of the pending claims under 35 U.S.C. first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention.

Regulatory Sequences

The Examiner alleges that the specification does not provide adequate written description for using any "regulatory sequences" to secrete an exogenous protein in the urine of a transgenic non-human mammal as claimed.

The Examiner states that the examples provided in the specification are limited to the production of transgenic mammals or proteins in the urine of transgenic mammals using the WAP or uroplakin promoter. Applicants respectfully disagree with the Examiner. The present application is not limited to the use of the WAP or uroplakin promoters since there is sufficient disclosure in the specification providing regulatory elements and sequences other than the WAP or uroplakin promoter that are useful to express proteins in cells of the urinary tract of the transgenic host with subsequent secretion into the host's urine. See pages 28 to 30, 41 and 42 of the specification.

To reiterate our previous arguments, applicants disclose other suitable regulatory sequences that are useful to drive expression of a protein that it is detectable in urine (see page 29 and 42 of the present application). Specifically on page 28, line 3 to page 29, line 31, the production of proteins in transgenic mammals using promoters and other regulatory sequences of kidney-, bladder- or urinary tract-specific genes is disclosed. Further, Examples 2 and 3 on pages 41 and 42 of the specification disclose gene constructs for expression in the urinary tract and secretion into the urine of the transgenic mammal.

In further support of this position, applicants enclose a declaration by Dr. Serguei Soukharev (See Appendix A), an employee of one of the assignee's, the American Red Cross, that discloses the preparation of gene constructs containing the 5' regulatory sequence of one of the disclosed proteins that are expressed in the urinary tract, a human uromodulin promoter, operably linked to erythropoietin (EPO) or to alpha-1-proteinase

inhibitor (a1-PI) genes. These constructs were made using the disclosure of the present invention to produce transgenic mammals that expressed EPO and a1-PI, which is secreted into their urine. Attachments 1-9 to the declaration provide the experimental data to support the statements made by Dr. Soukharev in his declaration.

Applicants maintain that the specification provides sufficient support and guidance for a skilled person to prepare the constructs containing a specific 5' regulatory sequence as claimed in claims 75 and 88 as is evidenced by Dr. Soukharev's declaration and supporting attachments. In this regard, the independent claims are limited to 5' regulatory sequences from proteins that are known to be associated with urinary tract tissue as disclosed on pages 20-27 of the specification. Indeed, Example 3 (page 42) of the present application states the 5' and 3' sequences of uromodulin, uroplakin,renin, erythropoietin, apolipoprotein E, aquaporin, nephrocalcin, osteopontin-k / uropontin, urinary kallikrein and urinary thrombomodulin genes are used in the present invention. Applicants have isolated the uromodulin promoter, sequenced it and prepared constructs for expressing EPO and a1-PI in transgenic mammals as evidenced by Dr. Soukharev's declaration.

While the specification does not teach "any and all" gene promoters, the regulatory regions of many genes expressed in the urinary tract have been sequenced and are known and available to the skilled artisan. Applicants contend that the skilled artisan is sufficiently skilled to select, isolate, manipulate and prepare constructs containing appropriate 5' and 3' regulatory elements of the described genes expressed in the urinary tract for use in preparing transgenic mammals that express a specific protein or peptide in cells in the urinary tract with the subsequent secretion into the urine. Both independent claims 75 and 88 require that the protein or peptide be expressed under the control of the recited 5' regulatory sequences and then secreted into the urine or detectable in the urine of the mammal, which limits the claims to 5' regulatory sequences that result in the expression of the protein or peptide in the cells of the transgenic mammal with the subsequent secretion of the protein or peptide into the urine of the mammal.

In regard to the 3' regulatory sequences result in the expression of the exogenous gene with subsequent expression into the urine, these sequences are contained in constructs that also contain the recited 5' regulatory sequences of claims 75 and 88 that result in the expression of the protein or peptide in the cells of the urinary tract of the mammals with the subsequent secretion into the urine of the non-human transgenic mammals. A skilled

person is capable of selecting and testing appropriate, functional 3' regulatory sequences without undue experimentation from known genes that encode proteins that are associated with the urinary tract of mammals. In view of the above arguments and Dr. Soukharev's declaration, it is requested that this rejection be withdrawn.

Proteins that degrade or detoxify organic material in the urine of transgenic non-human animals

The Examiner alleges that the specification fails to provide adequate written description for a transgenic non-human animal that expresses a protein that degrades food products or by-products thereof in its urine.

Applicants have amended claims 75 and 88 to recite the production of a protein or a peptide that is not limited to one that degrades and detoxifies organic material, which is supported on pages 30 and 31 of the specification. In view of this amendment to the claims, it is believed that this rejection is moot, and it is requested that this rejection be withdrawn.

Claims 5-7, 11-13. 15, 45-47, 51-57, 61-65 and 67-74

The Examiner alleges that while the specification is enabling for a transgenic non-human mammal whose genome comprises a transgene comprising a nucleic acid sequence encoding a protein operatively linked to a promoter that causes secretion of the protein into the urine of the transgenic mammal, where the protein is expressed and secreted into the urine of the transgenic non-human mammal and a method of producing the protein in the mammal with secretion in the urine, the specification does not enable any transgenic non-human mammal, any regulatory sequence or any protein that degrades or detoxifies organic material that is feces, urine, a microbe, chemical pollutant or a by-product thereof, a food product or by product thereof.

Transgenic non-human animals

The Examiner fails to find the arguments made previously persuasive to overcome the rejection that the specification does not enable making any "transgenic non-human animal" as broadly claimed. The Examiner maintains that applicants have not provided any evidence that the mouse WAP or uroplakin promoters have the equivalent functions in

mammals, birds and reptiles nor provided any promoters that direct expression of exogenous proteins in birds or reptiles. Further, the Examiner maintains that applicants have not provided any guidance to make transgenic reptiles, horses, dogs or cats.

Applicants maintain that the specification discloses methods than can be used to produce transgenic birds, reptiles, horses, dogs and cats by a person skilled in the art without undue experimentation. In particular, a horse is not such an unrelated species to other species, such as pigs, sheep, goats, and cows, that are known to be useful as transgenic hosts to express proteins. Applicants maintain that the specification is enabled for the scope of transgenic hosts, however, in an effort to expedite prosecution and to move the application toward allowance, applicants amend the claims to "non-human transgenic mammals" and to "a pig, sheep, goat, cow, rodent, rabbit or horse." It is requested that this rejection be withdrawn.

Regulatory sequences

The Examiner alleges that the specification does not enable using any regulatory sequences as broadly claimed to obtain secretion of the exogenous protein in the urine. Applicants wish to clarify the point that the regulatory sequences containing at least a promoter is responsible for obtaining expression of the exogenous protein in the cells of the urinary tract. After expression, a signal sequence of the expressed protein is responsible for the secretion of the expressed protein into the urine.

As noted above, the independent claims are limited to 5' regulatory sequences from proteins that are known to be associated with urinary tract tissue as disclosed on pages 20-27 of the specification. Applicants maintain that the specification provides adequate disclosure to identify, isolate and use 5' regulatory sequences from these know proteins. Thus, the claims are not directed to "any" regulatory sequences.

The Examiner states that secretion of the proteins into the urine is required. Both claims 75 and 88 require that the protein or peptide is either secreted into the urine or detectable in the urine. But as noted above, it is the promoter that results in the expression of the protein or peptide in the tissues of the urinary tract. These regulatory sequences do not result in the expression of the protein in the urine. But rather the proteins that are expressed contain signal sequences which are responsible for the secretion of these proteins into the urine.

Applicants maintain that the claims recite specific 5' regulatory sequences that are enabled by the present specification. It is requested that this rejection be withdrawn.

Proteins that degrade or detoxify organic material in the urine of transgenic non-human animals

The Examiner argues that the specification does not enable expressing any protein that degrades food products or by-products thereof in the urine of a transgenic non-human animal as broadly claimed. As applicants have noted above, claims 75 and 88 are amended to produce a protein or a peptide that is not limited to one that degrades and detoxifies organic material. These claims are supported by the specification as noted above. In view of this amendment to the claims, it is believed that this rejection is moot, and it is requested that this rejection be withdrawn.

3. **Rejections under 35 U.S.C. § 112, second paragraph**

Claims 6, 11, 45, 56, 69, 70, 73 and 74

These claims are rejected as allegedly not further limiting claims from which they depend. As all of these claims have been canceled and replaced with a new set of claims, it is believed that these rejections are now moot and should be withdrawn as to the pending set of claims.

4. **Rejections under 35 U.S.C. § 102**

Claims 6, 11, 12, 45, 47, 51, 54-57, 61, 64 and 65

The claims are rejected as anticipated by Sympson *et al.*("Sympson") because Sympson teaches a transgenic mouse whose genome comprises a sequence encoding stromelysin-1 operatively linked to the WAP promoter and WAP 3' untranslated region. The present claims are directed to a transgenic non-human mammal that produces a protein that is expressed in cells of the urinary tract cells and secreted into the urine. Claims 75 and 88 do not recite using a WAP promoter. The present claims are not anticipated by Sympson, and it is requested that this rejection be withdrawn.

Claims 6, 7, 11, 12, 13, 45-47, 51, 54-57, 61, 64, 65, 67, 69, 71 and 73

The claims are rejected as anticipated by Sun (WO 9693494) or Sun (U.S. Patent 5,824,543). The Examiner alleges that Sun teaches transgenic mice whose genomes comprise a sequence encoding β-galactosidase linked to the uroplakin promoter and obtaining expression of β-galactosidase in the urine and isolating the protein from the urine. The present claims do not recite using a uroplakin promoter operatively linked to an exogenous gene. Because Sun does not anticipate the present set of claims, it is requested that this rejection be withdrawn.

5.. **Rejection under 35 U.S.C. § 103**

Claims 6, 7, 11, 12, 13, 45-47, 51, 54-57, 61, 64, 65, 67, 69, 71 and 73

The claims are rejected as obvious over Sympson *et al.*("Sympson") in view of Lubon *et al* (U.S. 5,880,327), Sun (WO 9693494) or Sun (U.S. Patent 5,824,543) and Wen *et al.* ("Wen") The Examiner alleges that Sympson teaches the production of a transgenic mouse expressing rat stromelysin-1 cDNA under control of the WAP promoter and that WAP promoter directs expression inherently in the kidney resulting in protein production in the urine. The Examiner admits that Sympson does not suggest that stromelysin-1 gene could be isolated from the urine but applies Lubon or either of the Sun patents as teaching that proteins of interest could be isolated from urine of transgenic mice. The Examiner concludes that it would have been obvious to provide the transgenic mouse expressing stromelysin under the control of the WAP promoter as taught by Sympson and to isolate the protein from the urine as taught by Lubon, either of the Sun patents and Wen. The Examiner alleges that one of ordinary skill would have been motivated to isolate proteins from the urine of a mouse expressing proteins under the control of the WAP promoter because of the teaching of Wen that suggests isolating proteins from transgenic mice expressing proteins under the control of the WAP promoter to decrease the cost of making the protein.

The arguments made above against the anticipation rejections based on Sympson and either of the Sun patents, that the claims do not disclose a DNA construct disclosing a WAP promoter or a uromodulin promoter operatively linked to a protein or a peptide, are relevant to the obviousness rejection. Moreover, the secondary references do not cure the deficiencies in this rejection. None of the prior art suggests utilizing any of the recited

urinary tract protein regulatory sequences to express a protein or peptide in a transgenic mammal. In view of these arguments and an indication by the Examiner that previous claims 52, 53, 62 and 63 that recite specific 5' regulatory sequences are free of the prior, it is requested that this rejection be withdrawn.

Conclusion

In light of the foregoing amendments and remarks, applicants submit that all claims are in condition for allowance, and they solicit an early indication to that effect. Should the Examiner believe that further discussion of any remaining issues would advance the prosecution, he is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

November 5, 2001
Date

Jayme A. Huleatt
Reg. No. 34,485

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 030523/0141

In re patent application of
LUBON *et al.*

Group Art Unit: 1633

Serial No. 08/982,284

Examiner: M. Wilson

Filed: December 1, 1997

For: METHODS FOR THE DEGRADATION AND DETOXIFICATION OF ORGANIC MATERIAL USING URINE PRODUCED BY TRANSGENIC ANIMALS AND RELATED TRANSGENIC ANIMALS AND PROTEINS

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
Washington, D.C. 20231

I, Serguei Soukharev, hereby declare:

1. I am an employee of the Holland Laboratory of the American Red Cross, an assignee of the captioned application. I have been a member of the Plasma Derivatives Department, from August, 1999 to the present. I am presently employed as a Scientist I . Attached is my CV to further explain my experience and background. See Attachment 1.

2. I have personally performed or supervised the performance of experiments in which DNA constructs containing the human uromodulin (URO) promoter was operably linked to a DNA sequence that encodes a heterologous gene, such as the erythropoietin (EPO) gene, or the alpha-1-proteinase inhibitor (a1-PI) gene.

3. The URO-EPO construct was inserted into a plasmid and designated p416. See Attachment 2A. This plasmid was utilized to prepare transgenic mice that expressed EPO in their urine. The p416 plasmid was injected into fertilized mouse eggs (CD-1 donors). Eggs that survived were transferred to foster mothers. Seven transgenic founders tested positive for the presence of EPO DNA using a PCR test as shown in Attachment 2B.

4. Samples of the urine of several of the transgenic EPO founders were collected and EPO levels were estimated by ELISA as shown in Attachment 3. To detect the presence of EPO in the urine of the F1 generation, founder Epo 2, not shown in Attachment 4, and Epo 7 were bred. Urine samples from several mice from the F1 generation of Epo 2 and Epo7 were tested and these samples demonstrated that the F1 generation produced EPO in the urine as shown in Attachment 4.

5. The attached transgenic EPO experimental data demonstrates that the urine of transgenic mice transfected with the DNA construct encoding EPO under the control of the uromodulin promoter contains detectable levels of EPO.

6. The constructed Uro-a1-PI, referenced in paragraph 2 above, and shown in Attachment 5A, was utilized to prepare transgenic mice that expressed a1-PI in their urine. This construct was injected into fertilized mouse eggs (CD-1 donors). Eggs that survived were transferred to foster mothers. Thirteen transgenic founders tested positive out of 60 mice tested for the presence of a1-PI DNA using a PCR test. Results of 6 positive mice are shown in Attachment 5B.

7. Samples of the urine of several of the transgenic a1-PI founders were collected and the presence of a1-PI by Western blot analysis is shown in Attachments 6 and 7. Urine samples from several mice from the F1 generation of different founder lines were tested by Western blot analysis and the tested mice in the F1 generation produced a1-PI in the urine as shown in Attachments 8 and 9.

8. The attached transgenic a1-PI experimental data demonstrates that the urine from transgenic mice that were transfected with the DNA construct encoding a1-PI under the control of the uromodulin promoter contains detectable levels of a1-PI.

9. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 10-31-01

S. Soukharev

Serguei Soukharev

Attachment 1.

CV of Serguei A. Soukharev

Serguei A. Soukharev
10417 Montrose Ave, apt 202
Bethesda, MD 20814.
301 5309792 Home 301 7380490 Office
E.mail : Soukharev@excite.com

EDUCATION : 1989 **M.Sc Thesis,**. in Biochemistry (with honour), Samara State University,
Samara, Russia. Department of Biology, Major in Biochemistry.

1994 **Ph.D** (Biochemistry) Thesis: 'Regulation of granulocyte activity by blood factors'. Institute of Cell Biophysics,
Russian Academy of Sciences, Pushchino, Moscow region, Russia.

PROFESSIONAL EXPERIENCE:

1988-1989 Graduate student, Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Biological Center of Soviet Union,
Russian Academy of Sciences, Pushchino,
Moscow Region, Russia.

1989-1991 Research Assistant, Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Biological Center of Soviet Union,
Russian Academy of Sciences, Pushchino,
Moscow Region, Russia.

1991-1996 Research Associate I-II, Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Biological Center of Soviet Union,
Russian Academy of Sciences, Pushchino, Moscow Region, Russia.

1996-1998 Postdoctoral Fellow, Laboratory of Biochemistry and Methabolism,
NIDDK, NIH,. Bethesda, MD.

1998-1999 Postdoctoral Fellow, Clinical Neuroscience Branch,
NIMH, NIH, Bethesda, MD.

1999-2000 Research Fellow II at Holland Laboratory, American Red Cross

2000-current time

Scientist I, Leader of Transgenic group at Holland Laboratory, American Red Cross

RESEARCH EXPERIENCE :

Apoptosis and protein degradation

- Studies of cell activation, protein degradation by lysosomal proteases, protein biosynthesis and membrane expression. Stress reactions of cells:

Activation of programmed cell death or apoptosis. Role of serine proteases and their targets in the activation of the cell death. Superexpression of serine proteases in different cell lines.

Cre recombinase

- Gene Targeting, using Cre-recombinase for gene targeting application.
- Development of highly accurate and highly efficient system for gene replacement in ES cells by using double lox strategy.

Knock-out and gene function / regulation

- Asialoglycoprotein receptor gene; sequence, regulation, splicing, promoter search.
- Development of asialoglycoprotein knock-out mice. Role of MHL-1 gene in blood protein turnover.
- Development of the Gaucher disease model in mice. Replacement of normal mouse GC gene by human mutated version using homologous targeting in ES cells.

Transmissible spongiform encephalopathies.

- Generation of transgenic mice expressing of human prion protein in brain/blood on Prp knock-out background as a model for study of blood product related CJD infectivity. Two different models of mice expressing the Prion protein in brain and blood have been constructed. Study of sensitivity to CJD infection is underway.

- Development of diagnostic assay for detection of abnormal prion protein in tissue and body fluids.

Generation of transgenic animals expressing human proteins for biothecnological purposes.

- Gene cloning and generation of expressing vectors for expression in transgenic animals
- Generation of transgenic animals for expression of a1-antitrypsin into the milk/ urine
- Generation of transgenic animals for expression of FVIII into milk. Co-expression of heavy and light chain of factor VIII into the mouse milk.
- Generation of transgenic animals for targeting the EPO expression into the urine.

TEACHING EXPERIENCE.

- Practical Biochemistry, Summer course for student of Pushino, Biotechnological center, Pushchcino, Russia. 1992-1994.

-Supervising of Master Degree thesis, Biotechnological center, Pushchcino, Russia. 1994-1996

-Supervising and teaching of research assistants of Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry. 1994-1996

- Supervising and teaching of three research assistants at The American Red Cross, Holland Laboratory. 1999-2000.

- Supervising the transgenic group/facility 2000-2001

AWARDS: Fogarty Fellowship, 1996-1999
European PECO fellowship, 1994
Honor Master Degree, 1989

Society Membership: Society for In Vitro Biology, New York Academy of Sciences (1999- present time)

PRINCIPAL PUBLICATIONS:

1. Smirnov S.V., Sukharev S.A., Shadrin B.P., Sadovnikov V.B.1989. "Inhibition by normal blood serum of the reduction of nitroblue tetrazolium by phagocytic cells", (In Russian) Immunologia: 6: 35-39.

2. Smirnov S.V., Sukharev S.A., Dmitrieva E.S., Morgunova L.V., Sadovnikov V.B. 1990. "Inhibition of Neutrophils-Mediated Lysis of syngenic erythrocytes by components of blood serum and peritoneal fluid". Biomedical Science: 1: 481-486.

3. Sukharev S.A., Pleshakova O.V., Sadovnikov V.B. 1995. " Cytotoxicity of mouse neutrophils at site of inflammation in vivo." (In Russian) Doclady Rossiyska Akademia Nauk: 343: N3: 403-405.

4. Sukharev S.A., Smirnov S.V., Sadovnikov V.B. 1994. "Heat-Shock of neutrophils at site of inflammation. " (In Russian) Doclady Rossiyska Akademia Nauk: 335: N4: 515-518.

5. Sukharev S.A. Sadovnikov V.B. " Apoptosis of murine neutrophils at inflammation in vivo". 3th Euroconference on apoptosis in Cuenca, Spain. 21-26 October, 1995.

6. V.V.Obraztsov, D.G.Shekhtman, A.N.Sklifas, A.B.Gapeev, V.G.Safronova, N.K.Chemeris, Sukharev S.A.,Sadovnikov V.B. 1995 "Emulsion of perfluorochenmicals inhibit neutrophil activity." (in Russian). Doclady Rossiyska Akademia Nauk: .342: N6: 819-822.

7. Sukharev S.A., Pleshakova O.V., Moshnikova A.B., Sadovnikov V.B., Gaziev A.I. "Age- and Radiation-Dependent Changes in Carbonyl Content, Susceptibility to Proteolysis, and Antigenicity of Soluble Rat Liver Proteins". Comp. Bioch. and Physiol. Vol 116B N3 pp333-338. 1997

8. Pleshakova OV., Kytsyi M.P., Sukharev S.A., Sadovnikov V.B., Gaziev A.I. Study of protein carbonyls in subcellular fraction isolated from liver and spleen of old and y-irradiated rats. Mechanism of Ageing and Development.103(1998)45-55 1998.

9. Ivan Lefkovits, Johann Rudolf Frey, Lotte Kuhn, Vladimir Sadovnikov, Olga Pleshakova, Serguei Sukharev. Neutrophils as a source of putative restriction proteases. The Journal of Immunology, 158: 4908-4915. 1997.

10. Sergey A. Sukharev, Olga V. Pleshakova and Vladimir B.Sadovnikov. Role proteases in activation of apoptosis. Cell Death and Differentiation, 4, 457-462. 1997

11. Soukharev S, Miller JL, Sauer B. Segmental genomic replacement in embryonic stem cells by double lox targeting. Nucleic Acids Res 1999 Sep 15;27(18):e21

12. Soukharev S, Berlin W, Hanover JA, Bethke B, Sauer B. Organization of the mouse ASGR1 gene encoding the major subunit of the hepatic asialoglycoprotein receptor. Gene 2000 Jan 11;241(2):233-40

13. Soukharev S, B Sauer. New role of asialoglycoprotein receptors. MHL-1 knock-out mice. In manuscript.

14. Cervenakova, L., Soukharev, S., Yakovleva, O., McKenzie, C., Drohan, W.N., Bruce, M., Brown, P.: A rapid incubation period RIII mouse model of vCJD. Lancet (submitted).

PRESENTATIONS and CONFERENCES:

Soukharev S.A., Sadovnikov V.B. " Apoptosis of murine neutrophils at inflammation in vivo". 3th Euroconference on apoptosis. 21-26 October 1995 Cuenca, Spain.

Soukharev S.A., Le Y.Z., Miller J. and Brian Sauer. High frequency genomic targeting in ES cells by Cre recombinase. Conditional genetic technologies in the mouse. August 31-September 1998. Cold Spring Harbor, New York.

Soukharev S.A., Miller J., and Brianh Sauer. High frequency gene targeting by Cre recombinase in mammalian cells. Gene Therapy. September 22-27, 1998 Cold Spring Harbor. New York.

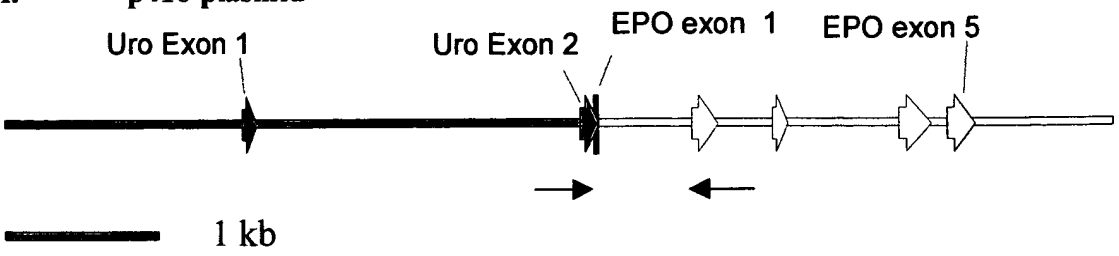
Cervenakova L., Soukharev S., Brown P. Toward the development of an assay for detection of pathological PRP isoform in blood. CHI meeting Transmissible Spongioform Encephalopathies. October 2-3, 2000.

GENE BANK SUBMISSION.

LOCUS AF182811 7590 bp DNA ROD 02-FEB-2000
DEFINITION Mus musculus asialoglycoprotein receptor major subunit gene, complete cds. ACCESSION AF182811 VERSION AF182811.1 GI:6851267
SOURCE house mouse. REFERENCE 1 (bases 1 to 7590)
AUTHORS Soukharev,S., Berlin,W., Hanover,J.A., Bethke,B. and Sauer,B.

Attachment 2. The map of p416 plasmid and PCR screening of founders.

A. **p416 plasmid**



Uromodulin part of the vector indicated by filled boxes, expressed gene (EPO) in open boxes. Only part of uromodulin promoter is shown.

B.

A. Primers used for PCR detecteion are indicated by arrows.
B. - and + positive and negative control. Correct PCR product is indicated arrow

Attachment 3.

Table 1. Expression of rhEpo in the urine of transgenic founder mice estimated by ELISA

Lines of transgenic mice		*ng/ml (mean ± SD)*
Epo 1	(1)	5.25 ± 0.38^b
	(2)	6.95 ± 0.68^a
Epo2		ND
Epo 3	(1)	0.44
	(2)	0.09
	(3)	0.25
	(4)	0.28
Epo 5	(1)	6.07 ± 0.47^b
	(2)	5.28 ± 0.37^b
	(3)	8.13 ± 0.37^b
Epo 6		NDT
Epo 7	(1)	0.57
	(2)	0.55
	(3)	0.22
	(4)	1.04
Epo 8		NDT

Experiment was carried out in triplicate[a] or in quadroplicate[b]
(1-4) - days of urine collection
NDT – not detectable (<25 pg/ml)
ND - not determined

Attachment 4.

Concentration of rhEpo in the urine of transgenic offspring (lines Epo2 and Epo7)

Mouse identification number and description		*rh Epo pg/ml in urine samples (mean ± SD)*
Control mice[a]		NDT
Transgenic mice	# 2266 (Epo 2) m,F1	164.4 ± 9.1[b]
	# 815 (Epo 7) f,F1	263.6 ± 36.6[b]
	# 811 (Epo7) f,F1	49.2 ± 14.0[b]

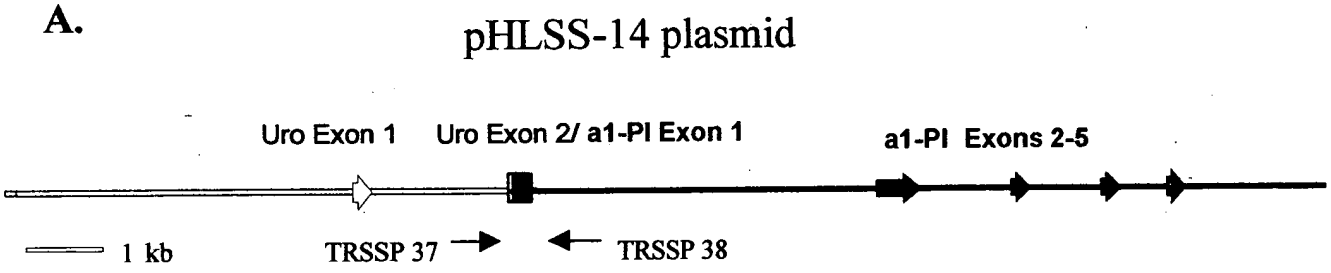
[a]three mice were tested
Experiment was carried out in triplicate[b]
NDT – not detectable (< 25 pg/ml)

Attachment 5. The map of pHLSS14 (Uro-a1 PI) and PCR screening of positive founders.

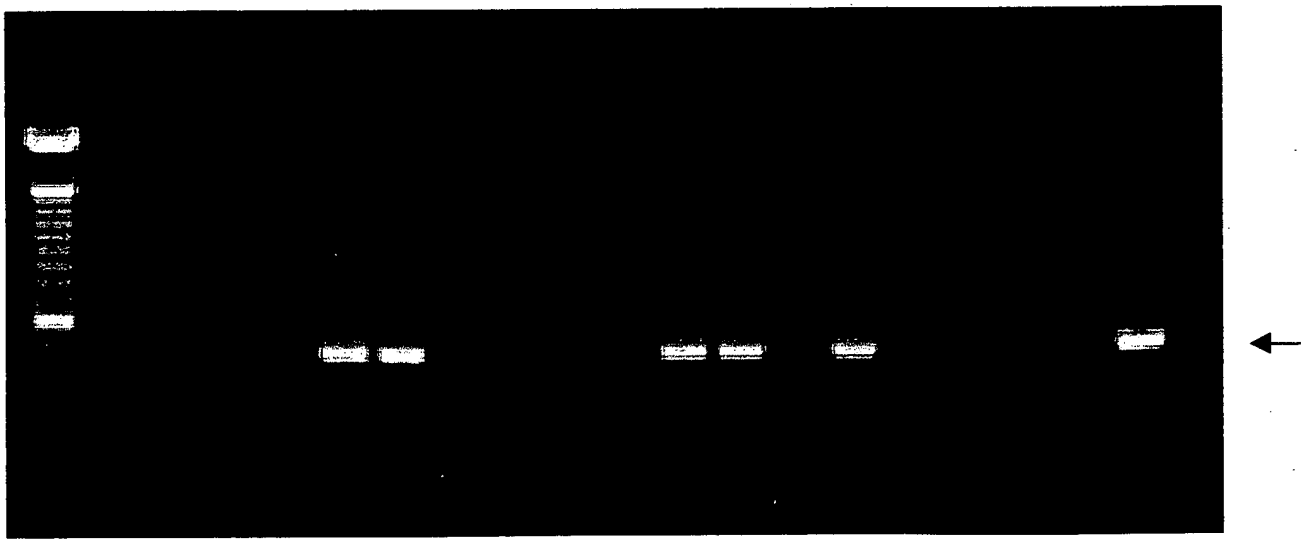
A. Arrows indicate location of primer used in diagnostic PCR. Uromodulin part of the vector indicated by open boxes, expressed gene (a1-PI) in closed boxes. Only part of uromodulin promoter is shown.

B. Detection of 5 positive founders is shown. Arrows indicate the correct PCR product.

A.



B.



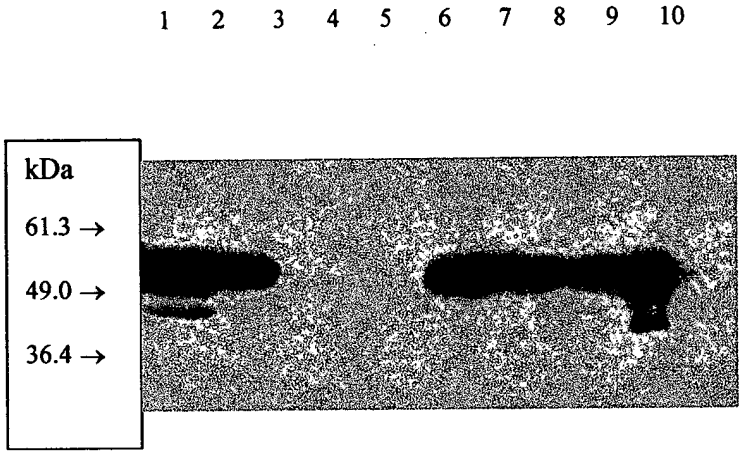
Attachment 6.

Expression level of rh a1-PI
in the urine of transgenic founder mice estimated by
Western blot (Limit of detection: <0.5 μg/ml)

ID #	Cage #	Sex	Approx. level of rhAPI expression (μg/ml)
3758	1	F	0.5 – 1
3759	2	M	0.5
3764	3	M	undetectable
3765	4	M	undetectable
3767	5	M	5 – 6
3317	6	F	undetectable
3319	7	F	2 - 3
3333	8	M	8 - 11
3342	9	M	5 - 6
3481	**10**	**M**	**20-25**
3486	11	M	undetectable
3491	12	F	undetectable
3495	13	F	undetectable

Attachment 7.

Detection of a1-PI in mouse urine.



Western blot analysis of rh a1-PI in the urine of transgenic founder mice. Samples of transgenic mice urine were desalted by Micro Bio-Gel P6 columns (BioRad), concentrated to dry and resolved in sample buffer.

1. Urine of the founder mouse # 3342 (50 µl)
2. Urine of the founder mouse # 3481 (18 µl)

3,4,5 Control mouse urine (50 µl)

6 Urine of the founder mouse # 3319 (50 µl)
7 Urine of the founder mouse # 3758 (50 µl)
8 Urine of the founder mouse # 3759 (50 µl)
9 Control human plasma (10 µl)
10 Control mouse plasma (10 µl)

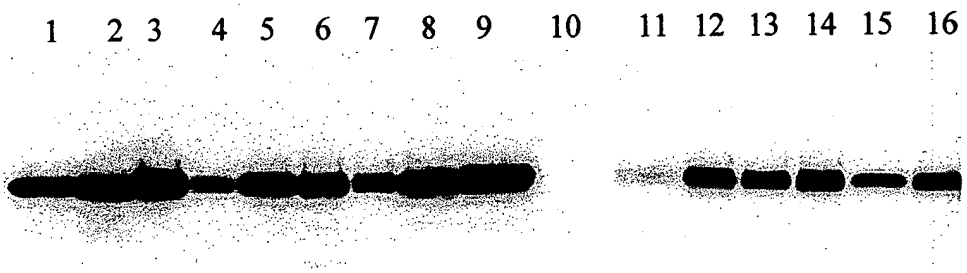
Attachment 8.

Expression level of rh a1-PI in the urine of transgenic offspring (F1 generation) estimated by Western blot

ID #	Sex	Line number of the founder mouse	Approx. level of rhAPI expression (μg/ml)
1209	F	3333	20
1210	F		2
1212	M		12
1213	F		12
1216	M	3342	7.5
1223	M		16
1230	M	3319	16
1232	M		1
1233	F		4
1234	M		4
3065	F	3758	4
3077	M	3759	2
3093	M	3317	2
1806		3481	ND
1807			40 - 60
1809			60 - 70
1813			ND
1815			ND
1816			10
1817			35 - 40
1819			20 - 30
1820			40 - 60
1824			30 - 35

Detection of a1-PI protein in F1 generation

Attachment 9.



1. Plasma-derived API spiked into control mouse urine (150 ng/lane)
2. Plasma-derived API spiked into control mouse urine (300 ng/lane)
3. Urine from mouse #1209 (line #3333)
4. Urine from mouse #1210 (line #3333)
5. Urine from mouse #1212 (line #3333)
6. Urine from mouse #1213 (line #3333)
7. Urine from mouse #1216 (line #3342)
8. Urine from mouse #1223 (line #3342)
9. Urine from mouse #1230 (line #3319)
10. Control mouse urine
11. Urine from mouse #1232 (line #3319)
12. Urine from mouse #1233 (line #3319)
13. Urine from mouse #1234 (line #3319)
14. Urine from mouse #3065 (line #3758)
15. Urine from mouse #3077 (line #3759)
16. Urine from mouse #3093 (line #3317)